ENDOCRINOLOGY, REGENERATION AND MATURATION IN NEREIS

D. W. GOLDING[1]

Department of Zoology, University of Bristol, England

The processes of growth and reproduction make heavy demands on the resources of an organism. It is not surprising to find that, as Clark (1962) pointed out, there is an incompatibility between growth and reproduction, though the degree of separation of the two processes varies considerably. In insects reproduction is delayed until molting and growth are completed. Herlant-Meewis (1962) remarked that the processes of scissiparity, involving somatic growth, and sexual reproduction do not occur together in the Naididae. According to Watson (1962), there is a different type of separation in Thysanura. Ecdyses and growth continue in the reproductive adult but reproduction and growth alternate during each instar. Limb regeneration occurs in the juvenile but not in the adult cockroach (Bodenstein, 1955; 1959) nor in adult decapods because of the essential nature of the molting hormone (Passano and Jyssum, 1963).

Among syllid polychaetes, specialized growth processes associated with reproduction lead to the development of stolons which bear the gametes. However, in more typical polychaetes, the growth rate declines as maturation proceeds (Clark, 1962; Clark and Clark, 1962; Clark and Scully, 1964).

Hormonal mechanisms effecting a separation between growth and reproduction have been demonstrated in nereids, and as in many other animals, the secretion of a juvenile hormone is involved. The supraesophageal ganglion, or brain, of nereids secretes a hormone or hormones which promote segment growth but inhibit maturation during the early stages of development (see reviews by Durchon, 1962; Clark, 1965; and Hauenschild, 1966). Immature nereids regenerate lost posterior segments. This segment growth is dependent on the presence of the brain hormone both for its initiation and its further progress (Casanova, 1955; Durchon and Marcel, 1962; Clark and Ruston, 1963b; Hofmann, 1966; Golding, 1967a, 1967b). However, Durchon (1965) has reported that 4-month-old *Perinereis cultrifera,* having about 50 segments, is able to regenerate several times over after the removal of the prostomium. Unpublished observations by the author indicate that very young *Nereis limnicola,* having about 20 segments, is unable to regenerate posterior segments in the absence of the prostomium. Possibly *Perinereis* differs from *Nereis* in this respect.

Several authors have noted the inability of mature *Nereis diversicolor* to regenerate segments (Stephan-Dubois, 1956; Clark and Ruston, 1963b; Scully, 1964). Clark and Ruston (1963b) and Scully (1964) investigated this phenomenon by designing experiments to test the following two possible explanations: (a) that

[1] Present address: Department of Zoology, University of California, Berkeley, California 94720.

mature animals do not regenerate because of a failure on the part of the supraesophageal ganglion to secrete the necessary hormone, or (b) that they do not regenerate because their tissues have become incapable of responding to the hormone.

Both investigations yielded results which were interpreted to mean that the first of these two explanations is the correct one.

The experiments described below were designed to reinvestigate the effect of maturation on regeneration and to shed light on the endocrine mechanisms involved in the control of these processes.

Materials and Methods

N. diversicolor was collected from the littoral zone of the River Avon, Bristol, England, and maintained in the laboratory in 50% sea water. Extirpation of the supraesophageal ganglion was carried out by the removal of the intact organ, together with the overlying epidermis. All experiments involved determination of the maximum oöcyte diameter of each of the animals used. This was accomplished by placing the animal on dry filter paper and puncturing the dorsal body wall about half-way along the length of the body with a fine glass capillary tube. The tip of the latter was ground on carborundum paper in order that it might cause as little damage as possible. Coelomic contents were forced into the tube by the internal hydrostatic pressure. A drop was blown out onto a coverglass which was inverted over a cavity slide. The maximum oöcyte diameter was measured by microscopic observation and the use of a calibrated micrometer eye-piece.

Further details, including those describing grafting techniques, have been given elsewhere (Golding, 1967a, 1967b).

Statistical significance of data was determined by the use of the *t*-test.

Results

Regenerative ability and maturity

In the first experiment, the regenerative ability of animals at various stages of maturity was investigated. Six groups of animals were used, each group consisting of 10 specimens, and each being at a different stage of maturity. The first group was made up of animals in which oöcytes (or spermatocytes) had yet to be shed into the coelom. The other groups consisted of animals whose maximum oöcyte diameters were 21–60 μ, 61–100 μ, 101–140 μ, 140–180 μ, and above 180 μ, respectively. The number of segments possessed by each animal was determined and the mean

Table I

Regenerative ability and maturity

Maximum oöcyte diameter (μ)	0	20–60	61–100	101–140	141–180	180+
Mean no. of segments, initially	67	72	79	80	83	90
No. of animals	10	10	10	10	10	10
No. surviving 21 days	10	8	10	9	9	9
Mean no. of regenerated segments	11.0	11.9	10.5	8.0	2.1	0
S. E.	0.8	0.5	0.9	1.5	0.8	—

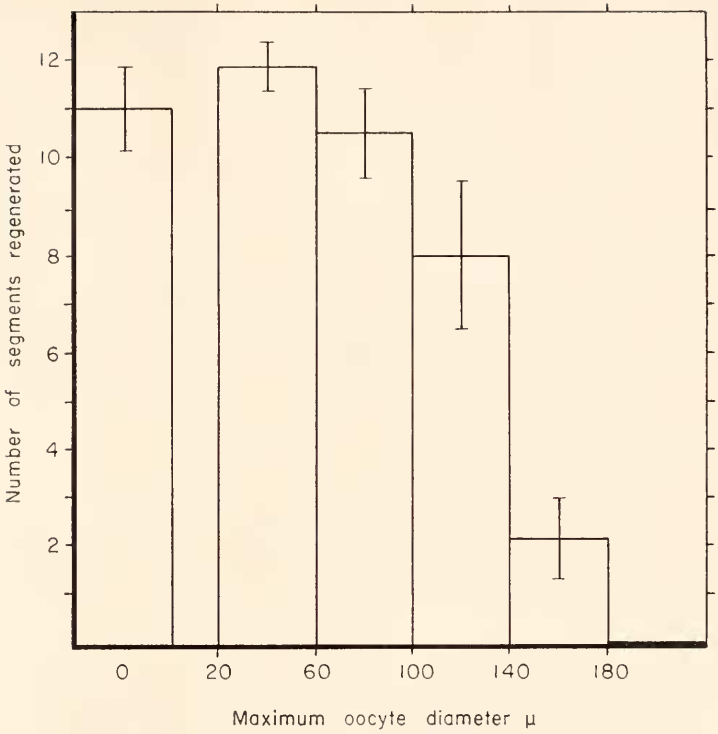

FIGURE 1. The regenerative ability of *Nereis diversicolor* at different stages of maturity.

number calculated for each group. Supraesophageal ganglia were allowed to remain *in situ*. Forty segments were amputated from each animal. After 21 days, the number of segments regenerated by each specimen was determined. The results are expressed in Table I and Figure 1.

One difficulty with respect to this experiment was that the amputation of 40 segments from each animal involved the removal of a greater proportion of the body of immature worms than that amputated from more mature ones, since the latter possess a greater number of segments. However, for the sake of simplicity, this factor was ignored.

It is clear that the regenerative capacity does not vary significantly during the stages of development represented by the different groups, until the maximum oöcyte diameter exceeds 140 μ. The mean number of segments regenerated by the group containing oöcytes 101–140 μ in diameter is less than that regenerated by the group with oöcytes 61–100 μ in diameter, but the difference is not statistically significant. However, the group of animals with oöcytes of 141–180 μ in diameter regenerated significantly fewer segments than less mature groups ($P < 0.01$), but significantly more than the most mature group, which regenerated no segments ($P < 0.05$).

Experiments were designed to determine whether the regenerative impotence of mature worms is due to an inability on the part of the supraesophageal ganglion

TABLE II

The secretory activity of the mature ganglion

Age of donors Age of hosts	Immature Immature	Mature Immature	Mature Mature*
No. of animals	20	20	30
No. surviving 21 days	19	18	20
Mean no.	9.5	4.3	0.1
S. E.	0.7	0.6	—

* Mature animals, with *in situ* ganglia; 17 out of 20 regenerated a pygidium.

to secrete regeneration hormone or to an inability of the body to respond to the hormone.

The secretory activity of the mature ganglion

In this experiment, 30 mature animals, each containing oöcytes > 180 μ in diameter, were first tested for regenerative ability. This was accomplished by amputating 40 segments from each. The ganglion was left *in situ*, since it has been shown previously (Golding, 1967a), that in animals capable of regenerating, it invariably induces more regeneration in this situation (in other words, the most stringent test for regenerative ability was applied to these animals). Another reason for leaving the ganglia *in situ* was that implanted ganglia are difficult to retrieve from the coeloms of mature animals, due to the opacity of the body wall. The jaws were removed and the animals kept together in aerated water for 21 days, after which they were examined.

As Table II (third column) shows, these animals were almost completely incapable of regeneration. Only 2 specimens regenerated one pair of parapodial rudiments each.

Forty immature animals (each with a maximum oöcyte diameter less than 120 μ), each 65–75 segments long, were divided into 2 groups of equal size. Forty segments and the ganglion were removed from each member of one group. The ganglia of the mature animals which had been tested and found to be incapable of regeneration were then transplanted into these decerebrate, tail-less hosts. The other group of immature animals acted as a control. Forty segments were removed from each, and each ganglion was transplanted into the coelom of another member of the group (so that no animal received its own ganglion as an implant). Both groups of hosts were kept together in one bowl of aerated water. They were separable into their respective groups due to distinctive parapodial clipping, carried out as described previously (Golding, 1967a). After 21 days, the number of segments regenerated was determined. The results are given in Table II.

The results show that ganglia removed from mature animals, incapable of regenerating, induce the proliferation of a significant number of segments in immature, tail-less hosts. However, they are not as effective as the ganglia of younger worms ($P < 0.01$).

In the second experiment, 50 immature animals, containing oöcytes < 120 μ in diameter, and having 65–75 segments, were divided into five groups of equal size.

Forty mature animals, containing oöcytes > 200 μ in diameter were divided into four groups of equal size. The supraesophageal ganglion and all but 35 anterior segments were removed from each of the first group of immature *Nereis*. These animals received implants of the ganglia of one of the groups of mature animals. The implanted ganglia were located and removed from the coelom after five days and replaced by ganglia freshly extirpated from mature donors. This procedure was carried out every five days so that each of these hosts had finally been subjected to the influence of four ganglia removed from mature animals, consecutively, each ganglion remaining in the coelom for five days after its initial extirpation.

The second group of immature hosts were prepared in the way described for the first group. They received implants of the ganglia implanted into, and removed from, that group. Thus each member of the second group was subjected to the influence of four ganglia, implanted and removed one after another. Each ganglion remained in the coelom of the host from the 6th to the 10th day after initial extirpation.

The ganglia were implanted into the third group of hosts after removal from the second group. In this way each member of the third group was subjected to the influence of four ganglia, consecutively, each ganglion remaining in the coelom from the 11th to the 15th day after its implantation into an immature host.

By transplantation of the ganglia from the third to the fourth group, the latter were subjected to their influence from the 16th to the 20th day after their extirpation.

The fifth group constituted a decerebrate control, all but 35 segments being removed as for the other groups. Mock operations were carried out every five days.

The results are given in Table III. The numbers of segments regenerated by the four groups of hosts into which ganglia were implanted do not differ significantly. The results show that ganglia of mature animals secrete regeneration hormone during the first five days after their implantation into immature hosts, and that the subsequent rate of secretion is no higher than that during this time.

The competence of the mature host

This aspect of the problem was investigated by an experiment involving transplantation of ganglia from immature donors into mature hosts.

The regenerative ability of 20 immature animals (the maximum oöcyte diameter not exceeding 120 μ), each 65–75 segments long, was tested by implanting each ganglion into the coelom of another member of the group, and the removal of 40

TABLE III

Transplantation of mature ganglia

Time in days after extirpation	1–5	6–10	11–15	16–20	Control
No. of animals	10	10	10	10	10
No. surviving	10	10	10	10	9
Segments regenerated	4.1	4.3	3.9	3.1	0.6
S. E.	0.4	0.4	0.6	0.6	0.3

TABLE IV

The competence of the mature host

Age of donors Age of hosts	Immature Immature	Immature Mature	Mature Mature
No. of hosts	20	19	20
No. surviving 21 days	19	14	15
Mean no.	8.5	0	0
S. E.	0.5	—	—

segments from each. They were kept together for 21 days, after which the number of segments regenerated was determined (Table IV, first column). These animals were thus shown to be capable of prolific regeneration of segments, and their ganglia to be very active, endocrinologically speaking.

Forty mature animals, each containing oöcytes at least 180 μ in diameter, were divided into 2 groups of equal size. The first had 40 segments and the ganglion removed from each member, and received implants of the ganglia of the young animals, each ganglion being retrieved from the coelom of its immature host.

Forty segments were also removed from each member of the other group of mature animals. The ganglion of each was transplanted into the coelom of another member of the group.

Both groups of mature hosts were kept together, in one bowl of aerated water, for 21 days.

The results are given in Table IV. They show that neither the control group nor hosts with ganglia from immature donors regenerated segments. A mature worm cannot be made to regenerate by the implantation of an immature ganglion.

Neurosecretion in the mature animal

Although ganglia excised from mature animals, which have lost the ability to regenerate, induce a significant amount of regeneration when implanted into immature, decerebrate, tail-less hosts, this does not necessarily mean that secretion of hormone occurs *in the mature animal.*

TABLE V

Neurosecretion in the mature animal

	Hosts	Grafts
No. of animals	30	30
No. surviving 21 days	36	26
No. showing no regeneration	25	11
No. regenerating 1 segment	0	7
2 segments	1	7
3 segments	0	1
Mean no.	0.1	0.9
S. E.	—	0.2

TABLE VI

Effects of transplanting immature ganglia into grafted Nereis

	Immature ganglia implanted		No ganglia implanted	
	Host	Graft	Host	Graft
Number of animals	10	10	10	10
No. surviving	10	10	9	9
Segments regenerated	2.3	2.4	0.8	1.3
S. E.	0.4	0.4	0.4	0.4

In this experiment, fragments of worm prepared from immature animals were grafted into mature hosts. The grafts originated from animals 65–75 segments long, containing oöcytes not more than 120 μ in diameter. The hosts contained oöcytes at least 180 μ in diameter. Forty posterior segments were amputated from each host, and at least 40 from each graft. After 21 days, the numbers of segments regenerated by hosts and grafts were determined.

The results are expressed in Table V. They show that the hosts were virtually incapable of segment regeneration (one specimen regenerated 2 pairs of parapodial rudiments) which would be expected in view of their maturity. The extent of regeneration in the grafts was rather less than that which occurs in decerebrate animals at a comparable stage of development (for comparison, see Golding, 1967a, Table 2).

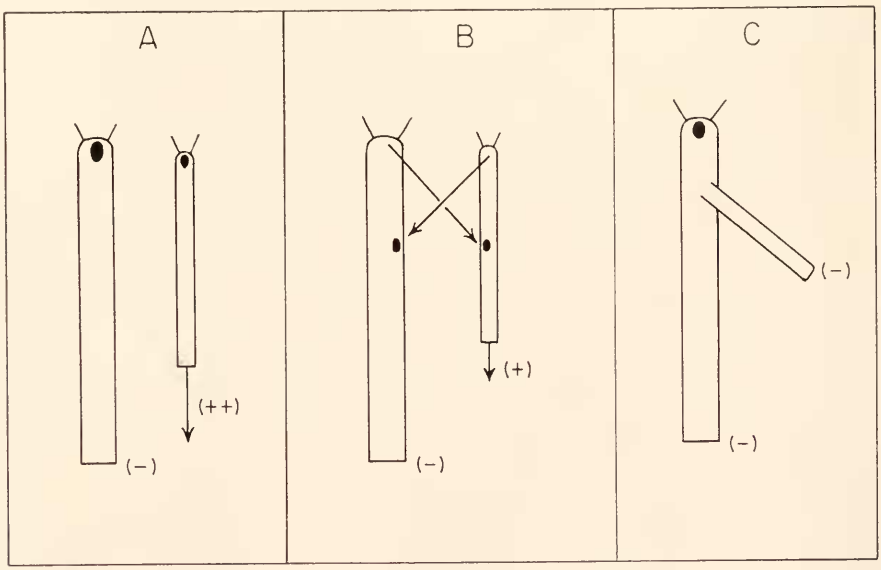

FIGURE 2. Diagrammatic representation of the principal experiments and their results. See text for detailed explanation.

In the second grafting experiment, 10 grafts were stitched into 10 hosts. Each graft consisted of segments 13–32 of an immature *Nereis*, containing oöcytes $< 120\,\mu$ in diameter, and having originally 60–75 segments. Each host was mature, containing oöcytes $> 200\,\mu$ in diameter. Forty segments were removed from each. Three ganglia originating from immature donors were implanted into each graft.

To provide a control, 10 comparable grafts were stitched into 10 mature hosts. Immature ganglia were not, however, implanted.

The two groups of grafted animals were kept for 21 days, after which the number of segments regenerated by hosts and grafts was recorded. The results are given in Table VI. They show that implantation of a number of immature ganglia induces a significant ($P < 0.05$) but very small amount of regeneration in hosts and grafts.

The principal experiments described in this paper, with their results, are diagrammatically represented in Figure 2.

Discussion

The results reported above are pertinent to four problems, namely: (a) The regenerative ability of animals at different stages of maturity. (b) The origin of differences in regenerative ability; that is, whether they are attributable to variations in the secretory activity of the supraesophageal ganglion or to the relative competence of the tissues. (c) The possibility that there is feedback mechanism involved, whereby maturation is both influenced by, and exerts an influence on, the secretory activities of the brain. (d) The relationship of the "regeneration hormone" and the "juvenile hormone."

When the maximum oöcyte diameter exceeds $140\,\mu$, there is a sharp decline in the ability of the animal to engage in regenerative growth. Mature animals containing fertile oöcytes at least $180\,\mu$ in diameter (Clark and Ruston, 1963a) are incapable of regenerating segments, though a small but complete pygidium is usually formed in each case. This conclusion is consistent with those drawn by Clark and Ruston (1963), Clark and Scully (1964) and Scully (1964).

The second problem was the subject of investigations by Clark and Ruston (1963b) and Scully (1964). They attributed the inability of older animals to regenerate to a virtual cessation of the secretory activities of the supraesophageal ganglion. The second experiment described above demonstrated that the ganglion does indeed decline in potency as maturation proceeds. However, it is still capable of secreting a significant amount of regeneration hormone (at least when implanted into an immature host). The donors used in this experiment contained oöcytes at least $180\,\mu$ in diameter. All were tested, with their ganglia *in situ*, with respect to their regenerative capacity, and found to lack the ability to regenerate segments.

Clark and Ruston (1963b) and Scully (1964) also asserted that the inability of older animals to regenerate is not due to incompetence on the part of the tissues to respond to the hormone. They reported that animals which are unable to regenerate normally will do so if ganglia from immature donors are implanted into them. The number of segments regenerated was small in the experiments of Clark and Ruston (1963b)—only 3 animals produced more than one pair of parapodial rudi-

ments. Scully (1964) obtained the regeneration of many segments. However, though the hosts were at least 90 segments long and control groups failed to regenerate, oöcyte diameters were not determined.

In the experiments reported above, only animals with oöcytes at least 180 μ in diameter were used as "mature hosts." Control groups were tested and found to be incapable of regeneration. Such hosts fail to regenerate significant numbers of segments after receiving implants of ganglia excised from immature worms. The latter (the actual specimens, not a control group) were tested and found to be capable of prolific segment regeneration before the ganglia were transplanted into the mature hosts.

Since ganglia from mature donors induce regeneration in immature hosts, whereas ganglia from immature donors do not cause mature hosts to regenerate, one might conclude that regeneration hormone is secreted in mature *Nereis* but that they are incapable of regenerating because of a deficiency on the part of the body. However, the results do not justify such a conclusion since the experiments involving implantation of ganglia provide no information about the effect of transplantation on the secretory activities of the ganglia. Though a ganglion from a mature animal secretes after implantation into an immature host, it may be inactive in the mature donor. Similarly, though a ganglion of an immature donor is demonstrably active in such an animal, it may be inhibited by transplantation into a mature host.

Comparison of the results obtained from two of the experiments indicates that the mature body may exert an influence on the secretory activities of the ganglion. The significant amount of regeneration ensuing when the ganglion of a mature worm is transplanted into an immature, decerebrate, tail-less host is in sharp contrast to the virtual absence of regeneration occurring in immature grafts implanted into mature hosts. Such grafts do not appear to be subjected to any hormonal influence whatsoever. It is possible that there is a feedback from the maturing animal to the ganglion, inhibiting the secretion of regeneration hormone. Such an influence might emanate from the ripening gametes, the neurosecretory cells of the ventral nerve cord, or from some other source.

A feedback mechanism affecting juvenile hormone secretion in *Perinereis cultrifera* was postulated by Durchon (1952) though his claim that the injection of mature öocytes into immature specimens precipitates maturation has not been substantiated. However, a comparable mechanism affecting the secretion of a hormone promoting gametocyte proliferation in *Arenicola* has been demonstrated by Howie and McClenaghan (1965).

Nevertheless, Hauenschild and Fischer (1962) are clearly correct in their view that the secretory activity of the ganglion is, to some extent, autonomous of the rest of the body. This is shown by the comparatively few segments induced in immature hosts by ganglia from mature donors. The amount of regeneration induced in mature hosts and their immature grafts by implanting several ganglia from immature donors is significant but very small. In comparison, immature grafts in large but immature hosts regenerate an average of 7–8 segments (Golding, 1967b)—without the addition of supernumerary ganglia. This probably indicates that the secretory activities of immature ganglia are not immediately, or completely, inhibited by the *"milieu"* of the mature host.

The fourth problem concerns the relationship of the regeneration-promoting hormone and the maturation-inhibiting hormone. Ruston (1964) thought that two distinct agents are likely to be involved in controlling the processes of regeneration and maturation because of the dissimilarity of the two processes. Clark and Ruston (1963b) investigated the problem and concluded that the two influences are not mediated by a single hormone since they found that oöcyte growth is not inhibited during regeneration, though Hauenschild (1966) reported that maturation is delayed after the loss of many segments in *Platynereis dumerilii*. Their reasoning depended on the assumption that regeneration hormone is secreted either after segment loss but not before, or in greater concentrations after segment loss. However, if the concentration of hormone remains steady throughout regeneration, as has been suggested by Golding (1967b), their results do not demonstrate the existence of two hormones.

The results obtained in this investigation are consistent with the idea that a single hormone influences both regeneration and maturation. The decline in the potency of the ganglion with respect to regeneration occurs mainly after the oöcytes have grown to 140 μ in diameter. Clark and Ruston (1963a) showed that decerebration causes little growth in oöcytes more than 140 μ in diameter in contrast to that induced in smaller oöcytes. From this it appears that the ganglion becomes less effective in promoting regeneration and inhibiting maturation at about the same stage in development.

It may be concluded that one hormone may be responsible for promoting growth (in intact and regenerating animals) and inhibiting maturation. This single hormone would ensure minimum competition between these two vital processes, stimulating growth but holding back maturation in the young animal. Upon its withdrawal, growth would cease and maturation would be precipitated.

This work was carried out during tenure of awards of the Science Research Council, U. K. The author is grateful to Professor R. B. Clark for his encouragement and constructive criticism; and to Mrs. Emily Reid who prepared the figures.

Summary

Normal and regenerative growth are partially separated from maturation, since during the later stages of maturation, growth does not occur. A mature animal almost invariably regenerates a pygidium but rarely regenerates segmental rudiments. The supraesophageal ganglion of a mature animal secretes less hormone than that of an immature animal, though it still induces a significant amount of regeneration when implanted into an immature, decerebrate host. The rate of secretion of such a ganglion is as great during the first five days after implantation into the host as it is subsequently. A single ganglion from an immature donor (in which it is known to be actively secreting) induces no regeneration when implanted into a mature host. Immature grafts, from which posterior segments have been removed, engage in virtually no segment regeneration when stitched into mature hosts. However, implantation of three ganglia removed from immature donors into each graft results in the formation of a significant but very small number of segmental rudiments in host and graft. These results suggest that there may be

a feedback from the maturing body, inhibiting the secretory activity of the ganglion. They are consistent with the suggestion that a single hormone secreted by the supraesophageal ganglion in immature *Nereis* both inhibits maturation and promotes growth in intact and regenerating animals.

LITERATURE CITED

Bodenstein, D., 1955. Contribution to the problem of regeneration in insects. *J. Exp. Zool.*, **129**: 209–224.

Bodenstein, D., 1959. The role of hormones in the regeneration of insect organs. *Scientia (Milan)*, **94**: 19–23.

Casanova, G., 1955. Influence du prostomium sur la régénération caudale chez *Platynereis massiliensis*. *C. R. Acad. Sci., Paris*, **240**: 1814–1816.

Clark, M. E., and R. B. Clark, 1962. Growth and regeneration in *Nephtys*. *Zool. Jb. (Physiol.)*, **70**: 24–90.

Clark, R. B., 1962. The hormonal control of growth and reproduction in polychaetes and its evolutionary implications. *Mem. Soc. Endocrinol.*, **12**: 323–327.

Clark, R. B., 1965. Endocrinology and the reproductive biology of polychaetes. *Oceanogr. Mar. Biol. Ann. Rev.*, **3**: 211–255.

Clark, R. B., and R. Ruston, 1963a. The influence of brain extirpation on oogenesis in *Nereis diversicolor*. *Gen. Comp. Endocrinol.*, **3**: 529–541.

Clark, R. B., and R. Ruston, 1963b. Time of release and action of a hormone influencing regeneration in the polychaete *Nereis diversicolor*. *Gen. Comp. Endocrinol.*, **3**: 542–553.

Clark, R. B., and U. Scully, 1964. Hormonal control of growth in *Nereis diversicolor*. *Gen. Comp. Endocrinol.*, **4**: 82–90.

Durchon, M., 1952. Recherches expérimentales sur deux aspects de la reproduction chez les annélides polychètes: l'epitoquie et la stolonization. *Ann. Sci. Nat., Zool. et Biol. Animal*, **14**: 119–206.

Durchon, M., 1962. Integration of reproductive functions. I. Neurosecretion and hormonal control of reproduction in Annelida. *Gen. Comp. Endocrinol.*, **2**: suppl. 1; 227–240.

Durchon, M., 1965. Sur l'évolution phylogénétique et ontogénétique de l'epitoquie chez les Néréidiens (Annelides Polychetes). *Zool. Jb. (Physiol.)*, **92**: 1–12.

Durchon, M., and R. Marcel, 1962. Influence du cerveau sur la régénération postérieure chez *Nereis diversicolor*. *C. R. Soc. Biol.*, **156**: 661–663.

Golding, D. W., 1967a. Neurosecretion and regeneration in *Nereis*. *Gen. Comp. Endocrinol.*, **8**: 348–367.

Golding, D. W., 1967b. Regeneration and growth control in *Nereis*. *J. Embryol. Exp. Morph.*, (in press).

Hauenschild, C., 1966. Der hormonale Einfluss des Gehirns auf die sexuelle Entwicklung bei dem Polychaeten *Platynereis dumerilii*. *Gen. Comp. Endocrinol.*, **6**: 26–73.

Hauenschild, C., and A. Fischer, 1962. Neurosecretory control of development in *Platynereis dumerilii*. *Mem. Soc. Endocrinol.*, **12**: 297–312.

Herlant-Meewis, H., 1962. In discussion following Clark, 1962.

Hofmann, D. K., 1966. Untersuchungen zur Regeneration des Hinterendes bei *Platynereis dumerilii*. *Zool. Jb. (Physiol.)*, **72**: 374–430.

Howie, D., and C. McClenaghan, 1965. Evidence for a feedback mechanism influencing spermatogonial division in the lugworm (*Arenicola marina*). *Gen. Comp. Endocrinol.*, **5**: 40–44.

Passano, L. M., and S. Jyssum, 1963. The role of the Y-organ in crab proecdysis and limb regeneration. *Comp. Biochem. Physiol.*, **9**: 195–213.

Ruston, R., 1964. Investigations on the endocrinology of the polychaete *Nereis diversicolor*. M.Sc. Thesis, University of Bristol, U. K.

Scully, U., 1964. Factors influencing the secretion of regeneration-promoting hormone in *Nereis diversicolor*. *Gen. Comp. Endocrinol.*, **4**: 91–98.

Stephan-Dubois, F., 1965. Évolution du régénérat caudal de *Nereis diversicolor* après irradiation régionale aux rayons-X. *Bull. Soc. Zool. France*, **81**: 199–207.

Watson, J. A., 1962. In discussion following Clark, 1962.